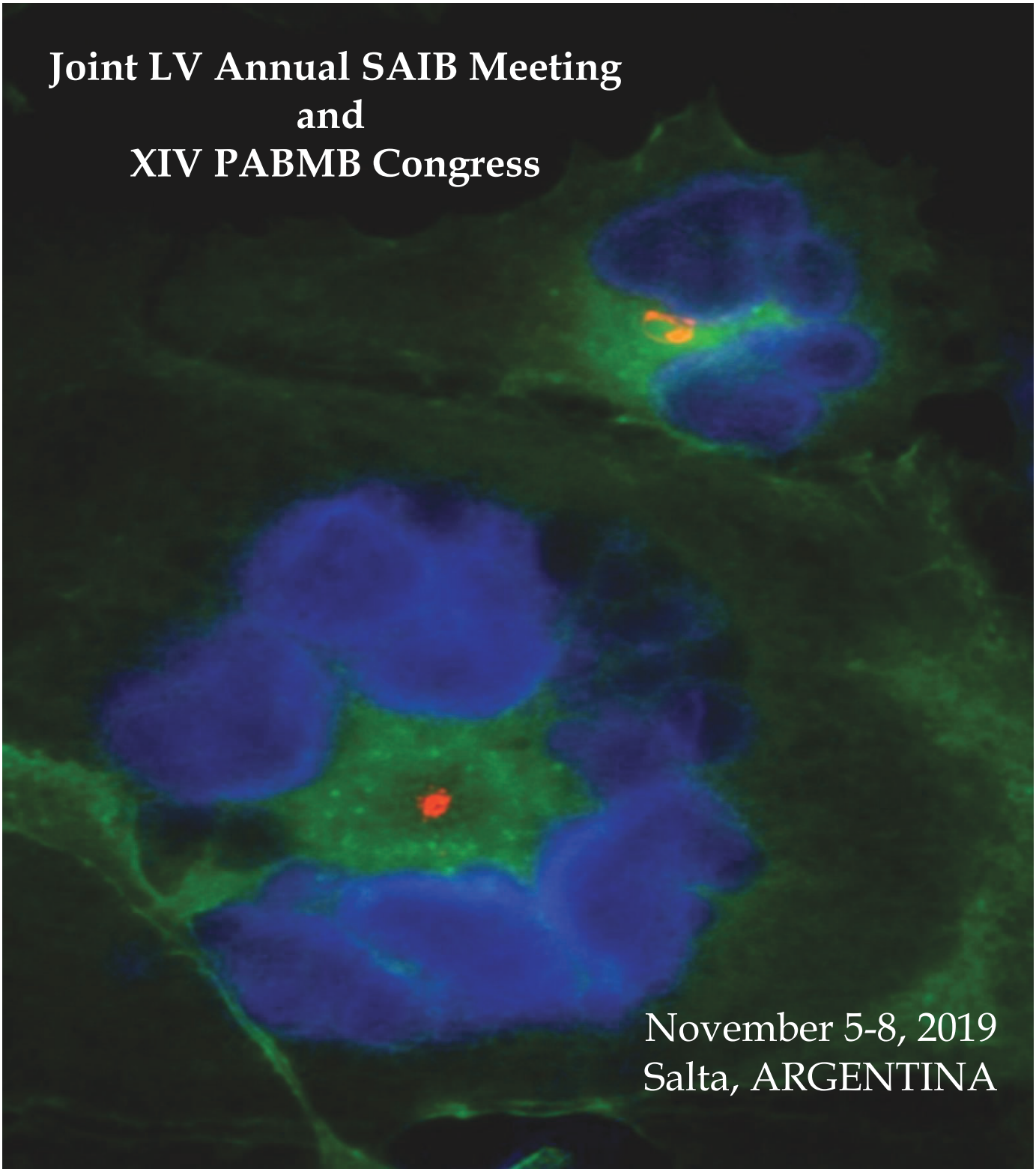
Joint LV Annual SAIB Meeting and XIV PABMB Congress



November 5-8, 2019
Salta, ARGENTINA

Tunicamycin has no effect on UPR in rapamycin treated cells. We analysed autophagy, a process that produces an increase in the intracellular amino acid pool. We observed that *tor1* and *leu3* cells present higher autophagy than wild type and that the addition of amino acids reduces the autophagic activity in all genotypes tested. In order to assess a link between UPR activation and chronological life span (CLS), we measured CLS using the colony forming unit spot assay. Longevity was affected by growth conditions and genotypes assayed; however we could not establish a direct correlation between UPR and CLS in all genotypes. Finally, we measured *GCN4* expression as a marker of protein synthesis and we found that it decreases in cells lacking Tor1 and wild type cells treated with rapamycin whereas increases in *leu3* cells. Altogether these results allow us to conclude that the addition of amino acids or the use of a rich nitrogen source activates UPR and that TORC1 and Leu3 are required in this process. We demonstrate that TORC1 and Leu3 are also involved in autophagy, amino acid biosynthesis and longevity but in some of these processes they act in an opposing manner.

ST-P02

NOVEL FUNCTION OF THE TRANSCRIPTION FACTOR UGA3 AS AN ACTIVATOR OF BRANCHED-CHAIN AMINO ACID PERMEASE *BAP2* GENE EXPRESSION

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Transcription regulation of most genes in yeast occurs at the level of activation, i.e. the basal level of expression is very low and increased transcription requires gene-specific transcription factors allowing the recruitment of basal transcriptional machinery. *Saccharomyces cerevisiae**BAP2*gene encodes the permease responsible for the major part of leucine, valine and isoleucine uptake, amino acids that this yeast can use as nitrogen sources. Moreover, *BAP2* expression is known to be induced by the presence of amino acids such as leucine. However, *BAP2*regulation by nitrogen source quality has rendered controversial results and remains unclear.In this context, we analyzed the transcriptional regulation of *BAP2* in response to extracellular leucine in the presence of both a poor and a rich nitrogen source. Our results show that *BAP2* expression is inducible by leucine in the poor nitrogen source proline, and that *BAP2* expression is constitutive in the rich nitrogen source ammonium, with high values unaltered by the addition of leucine. We also demonstrate here that an active SPS pathway is necessary for *BAP2* expression in both nitrogen conditions and in the presence or absence of the inducer leucine. Transcription factors Leu3, Gcn4 and Dal81are also involved in *BAP2* regulation in both a direct and an indirect way depending on the quality of the nitrogen source. We further demonstrate here that a physical interaction occurs between the transcription factorUga3and the regulatory region of the *BAP2* gene, which leads to a strong positive regulation. We thus conclude that the transcription factors involved in *BAP2* regulation affect its expression to different extents depending on the quality of the nitrogen source. We also demonstrate for the first time that Uga3, until now known as a transcriptional activator responsible for the induction of gamma-aminobutyric acid (GABA) genes, is one of the main positive regulators of *BAP2* transcription. So we found that*BAP2*, a gene expressed under environmental conditions quite different from those of *UGA* expression, is also regulated by Dal81, Uga3 and Leu3 factors together with Gcn4 and Stp1/2.

ST-P03

RELATIONSHIP BETWEEN THE GAAC AND TORC1 PATHWAYS WITH THE EXPRESSION OF AMINOACID PERMEASES ALONG AGING IN BUDDING YEASTS

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Protein biosynthesis, lifespan and autophagy induction are processes where the transcription factor Gcn4 has been described as an important regulator in the budding yeast *Saccharomyces cerevisiae*. This factor is mainly induced when amino acid starvation occurs. Several Gcn4 target genes belong to the GAAC (General Amino Acid Control) or to the TORC1 (Target Of Rapamycin) pathways. Important proteins that are downstream targets of these regulatory pathways are the amino acid permeases that internalize amino acids through the plasmatic membrane. Uga4 and Bap2 are two amino acid permeases induced by their substrates, γ-aminobutyric acid (GABA) and the branched amino acid leucine, respectively. The aim of this work was to analyse the transcriptional regulation of these permeases and *GCN4* in cells deficient in the GAAC (*gcn4Δ* and *leu3Δ* cells) and TORC1 (*tor1Δ*, *gln3Δ* and *sch9Δ* cells) pathways, grown in the absence and in the presence of amino acids during growth and along aging. For this purpose we used the lacZ reporter gene assays. We observed that in wild type cells *GCN4* expression decreases after cells reach the stationary growth phase; and when TORC1 is inhibited by rapamycin, *GCN4* expression slightly decreases and then remains constant along time. In *leu3Δ* cells high levels of *GCN4* expression are detected at the exponential phase and they drastically diminish during growth; but when these cells are treated with rapamycin *GCN4* remains high. The analysis of *GCN4* expression in cells deficient in *GLN3* and *SCH9*, two downstream targets of TORC1, showed that this expression varies in opposite direction. These results suggest that *GCN4* expression along growth depends on the TORC1 pathway. We also found that the expression of Uga4 and Bap2 permeases is differentially regulated in wild type cells. Whereas *UGA4* expression increases along cells aged, even though it has been described that protein translation is generally reduced in aged yeast cells, *BAP2* remains low, and Gcn4 seems to be needed for *UGA4* full expression. The deficiency in genes of the TORC1 pathway does not affect *UGA4* expression, and only in *tor1Δ* cells we observed an increase in *BAP2* expression. Further research must be done to understand how Gcn4, Uga4, Bap2 and other permeases such as *GAP1*, a general amino acid permease, are regulated during aging both at transcriptional and translational levels by the nutrient sensing signaling pathways TORC1 and GAAC.

ST-P04

A REGULATORY AXIS CONNECTING PKCA AND ZEB1 MODULATES EPITHELIAL-MESENCHYMAL TRANSITION AND INVASIVENESS OF BREAST CANCER CELLS

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The Epithelial-Mesenchymal Transition (EMT) is an essential program of embryogenesis and tumor progression, ZEB1 is a master regulator of the EMT. While extensive evidence confirmed the importance of ZEB1 as an EMT transcription factor that promotes tumor invasiveness and metastasis, little is known about its regulation. The aim of this work was to explore the signaling pathways that regulate ZEB1 levels and functionality, and how this regulation impacts on the dynamics of the EMT in cancer cells. We screened for potential regulatory links between ZEB1 and multiple cellular kinases. Our preliminary in silico studies revealed a plethora of potential phosphorylation sites for several kinases. Due to this level of complexity, we decided to follow up this analysis using ZEB1 deletion mutants (ZD1-HD and NZEB1), these constructs represent 60% and 10% of the full-length protein, respectively, and both retain the capacity to repress the E-cadherin promoter in cells, as determined with a luciferase reporter assay in cells. Intriguingly, we found that NZEB1 is enriched in PKC-specific sites and a substrate of p-PKC antibodies in cell extracts, thus suggesting an unforeseen regulatory role of PKC kinases on ZEB1 biology. Our initial experiments showed that NZEB1 and full length ZEB1 (ZEB1-FL) levels were actively reduced when NMuMMG-NZEB1 or MDA-MB-231cells were treated with the pharmacological inhibitors of PKCs GF109203X and Gö69761. To study the penetrance of this phenotype with ZEB1-FL, we investigated the levels of three well-known PKCs paralogs (α, δ and ε), ZEB1 and EMT makers in a group of 9 breast cancer cell lines. Strikingly, we found that PKCα and ZEB1 had a significant positive correlation, being both proteins overexpressed in cell lines with more aggressive phenotypes. Subsequent validation experiments using siRNAs against PKCα in MDA-MB231 cells revealed that its knockdown leads to a concomitant decrease in ZEB1 levels, while ZEB1 knockdown had no impact on PKCα levels. Remarkably, PKCα-mediated downregulation of ZEB1 recapitulates the inhibition of mesenchymal phenotypes, including inhibition in cell migration and invasiveness. These findings were extended to an in vivo model, by demonstrating that the stable knockdown of PKCα using lentiviral shRNAs markedly impaired the metastatic potential of MDA-MB-231 breast cancer cells. Conclusion: We demonstrated for the first time that the PKCα signal transduction pathway regulates the biological function of ZEB1, defining a novel regulatory axis of the EMT program in breast cancer cell lines, which might stimulate the evaluation of PKC inhibitors for metastatic breast cancer therapy

ST-P05

VDR AGONISTS TRIGGER WNT/B-CATENIN DOWNREGULATION IN A CELLULAR MODEL OF KAPOSI'S SARCOMA

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We have previously shown that $1\alpha,25(OH)_2D_3$ exerts antiproliferative effects in endothelial cells stable expressing Kaposi's Sarcoma-associated Herpesvirus G Protein-coupled Receptor (vGPCR) through NF-κB pathway inhibition and apoptosis induction. β-catenin, a multifunctional protein, is required for cell-cell adhesion and gene expression regulation in response to Wnt. Aberrant activation of this pathway provokes β-catenin accumulation in the nucleus and induces cell proliferation. Since it is well documented that vGPCR activates the canonical Wnt/β-catenin signaling pathway, we investigated if β-catenin and its target genes are regulated by $1\alpha,25(OH)_2D_3$. Firstly, Western blot studies showed an increase in β-catenin protein levels, which were not affected by the presence of Actinomycin D, a transcription blocker, after $1\alpha,25(OH)_2D_3$ (10 nM, 24 h) treatment. Secondly, β-catenin localization, investigated by immunofluorescence and subcellular fractioning techniques, was increased in the nucleus and plasma membrane. This event was accompanied by an increase in VE-cadherin in the cell membrane. Furthermore, a β-catenin/VDR interaction was observed by co-immunoprecipitation, which correlated with a decrease in the expression levels of β-catenin target genes *c-myc, cyclin D1* and *MMP-9* mRNA after $1\alpha,25(OH)_2D_3$ (10 nM, 48 h). Finally, Dkk-1, the extracellular inhibitor of Wnt/β-catenin pathway, elicited an initial upregulation of mRNA expression accompanied by lower β-catenin protein levels (0.5-1 h). Altogether, β-catenin/VDR interaction may account for non-transcriptional accumulation of β-catenin protein levels and downregulation of its target genes in response to $1\alpha,25(OH)_2D_3$

ST-P06

DIFFERENT SUGARS EXCERT NOT OVERLAPPING EFFECTS ON THE GROWTH REGULATION OF *ARABIDOPSIS THALIANA*

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Sugars are not only energy sources, but also important molecular signals that regulate growth, development, and response to environmental stresses. In plants, sucrose (Suc) is the main sugar for systemic transport and a specific signaling pathway for this disaccharide has been described. Although, the underpin sensor and mechanism are still unknown. Besides, it was proposed than Suc could be an antioxidant metabolite, especially when it is present at high concentrations, acting as ROS scavenger. On the other hand, glucose (Glc) is sensed through dependent and independent mechanisms of hexokinase. To gain insights into these subjects, we studied the effects of different sugars on the activation of root meristems (RAM) and redox homeostasis in *Arabidopsis thaliana*. The results showed that exogenous Suc and Glc differently induced the root growth, and fructose in a minor extent, depending on their concentrations. In contrast, trehalose (Tre) was not able to affect the root length. Besides, endogenous increment of Tre/Tre-P levels using a trehalase inhibitor, validamycin-A, did not induce the quiescent meristem. To test the putative protective role of sugars, we analyzed the effect of the different sugars on the inhibition of RAM produced by oxidative stress. The data indicated that only the addition of Suc could override the negative effect of methyl-viologen on root growth. Moreover, we measured the activity of Target Of Rapamycin (TOR), a master integrator of external and internal signals that regulates growth and development in eukaryotes. Results revealed differences on the kinase activity in seedlings grown with distinct sugars. In summary, we